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Case Report

Pneumoproteins as markers of paraquat lung injury: A clinical case

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Astract

Objective: To describe the changes in lung-specific secretory proteins in biological fluids in a fatal case of paraquat ingestion and to present immunostaining data obtained on postmortem lung tissue specimens.
Methods: A 20-year-old man committed suicide by ingesting 100 ml of a 20% paraquat solution. Surfactant associated proteins A (SP-A), B (SP-B) and Clara cell 16 kDa protein (CC16) were determined in the serum and on broncho-alveloar lavage performed 18 h after admission. Renal failure progressed rapidly and the patient died from refractory hypoxia. Immunostaining studies using antibodies directed against CC16, SP-A and SP-B were performed on postmortem lung tissue specimens.
Results: Serum CC16 seemed to increase gradually with the progression of renal impairment. Serum SP-A and SP-B levels increased before any significant changes in pulmonary gas exchanges. The immunostaining study showed that the labeling for SP-A and SP-B was reduced or absent following paraquat toxicity, while Clara cells were relatively preserved.
Conclusions: The elevation of serum CC16 with paraquat toxicity is probably mainly related to a reduced renal clearance. The increase of serum SP-A and SP-B could reflect an increased lung to blood leakage, independently of the alteration of the renal function.

Keywords: Clara cell 16 kDa protein; Paraquat; Acute lung injury; Surfactant associated proteins A and B

1. Introduction

Paraquat is a well known toxic agent that is still responsible in humans for acute respiratory failure and death when ingested for suicidal purpose.[1] The severity of paraquat poisoning is usually assessed by the determination of serum paraquat concentration and alteration of pulmonary gas exchanges.[2,3] Pneumoproteinemia is a recent concept in the assessment of lung disorders.[4,5] It refers to the assay in serum of some lung-specific secretory proteins, such as the bronchiolar Clara cell 16 kDa protein (CC16) and alveolar surfactant-associated proteins A and B (SP-A and SP-B). The levels of these proteins in the bloodstream have been shown to provide an non-invasive tool to evaluate the integrity of the broncho-alveolar/blood barrier. To our knowledge, this innovative approach has previously not been applied to evaluate paraquat-induced lung injury. The objective of this work was to document, from a case of fatal paraquat poisoning, the changes in some of these promising biological markers following paraquat exposure.

2. Case report

A 20-year-old non-smoker man without medical past history was referred to the emergency department 90 min after having voluntarily ingested more than 100 ml of paraquat (Gramoxone® 20%). The patient was agitated and vomited a bluish liquid. As gag reflex was absent, it was decided to intubate the patient in order to prevent aspiration pneumonia. He was administered 0.21 O_2 by continuous positive airway pressure ventilation under mild sedation. The admission chest-X-ray examination was not

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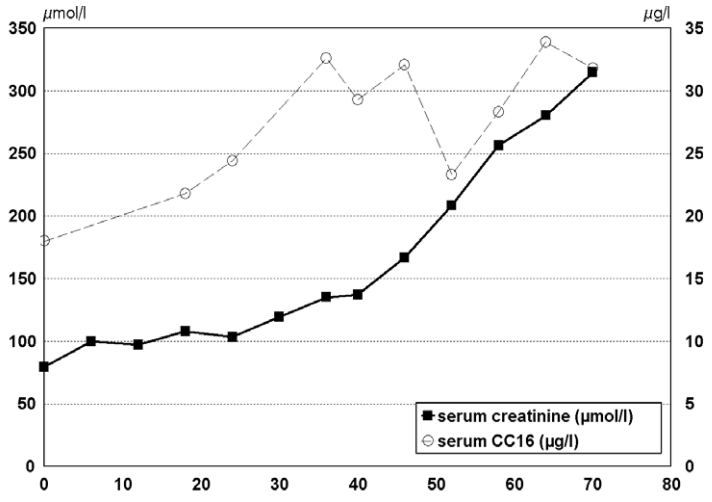
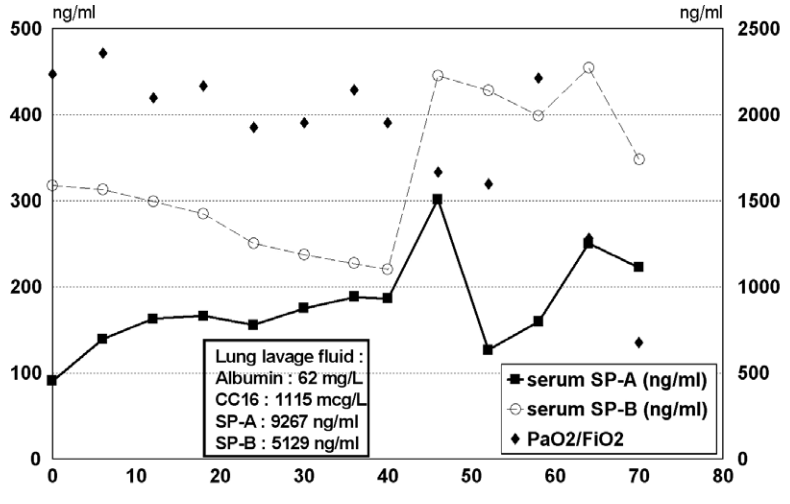
contributive. Biological data were irrelevant, urine output and renal function were preserved. Plasma paraquat level on admission (90 min after ingestion) was 46 μg/ml. The paraquat level was 15.4 μg/ml after 3 h, and 6.7 μg/ml after 7 h. According to the standard prognostic curves, these values are extremely high and indicate uniformly a poor prognosis. Under insistence of the relatives, it was decided to propose maximal therapy. Hemodialysis combined to hemoperfusion was performed. Drug therapy included i.v. cyclophosphamide (1 g/day), methylprednisolone (1 g/day) and *N*-acetylcystein (12 g/day). Renal function progressively deteriorated. Gas exchanges worsened 80 h after admission and chest-X-ray examination revealed bilateral infiltrates rapidly progressing to adult respiratory distress syndrome (ARDS). The patient died 12 h later and an autopsy was obtained. Both lungs were oedematous and congestive especially at the bases. The interstitium was largely infiltrated by a mixed inflammatory infiltrate. Hyaline



	0	6	12	18	24	30	36	40	46	52	58	64	70
serum creatinine (μmol/l)	79,6	99,9	97,2	107,8	103,4	119,3	135,2	137	167	208,6	256,4	280,2	314,7
serum bêta-2 microglobulin (mg/l)	0,76	0,82	0,86	0,75	0,85		0,97	0,9	1,21	1,39	1,82	1,89	2,44
serum cystatin-C (μg/l)	852	924	1052	924	1151	1322	1576	1407	1733	1753	2338	2088	3126
serum CC16 (μg/l)	18			21,8	24,4		32,6	29,3	32,1	23,3	28,3	33,9	31,8

Fig. 1. Evolution from hospital admission (90 min after exposure) of serum levels of creatinine, β-2 microglobulin, cystatin-C and Clara cell protein (CC16) in the paraquat poisoned patient.



	0	6	12	18	24	30	36	40	46	52	58	64	70
serum SP-A (ng/ml)	91,1	139,4	163,2	166,2	155,9	175,3	188,5	186,5	302	127,1	160	250,6	222,6
serum SP-B (ng/ml)	1589	1565	1497	1424	1252	1187	1136	1101	2227	2141	1993	2273	1740
PaO2/FiO2	447	471	419	433	385	390	428	390	333	319	442	256	135

Fig. 2. Concentrations of the Clara cell protein (CC16), albumin, surfactant-associated protein A (SP-A), surfactant-associated protein B (SP-B) in the broncho-alveolar lavage fluid of the paraquat poisoned patient, and evolution over time of SP-A and SP-B in the serum. The time-course is indicated from admission, 90 min after exposure.

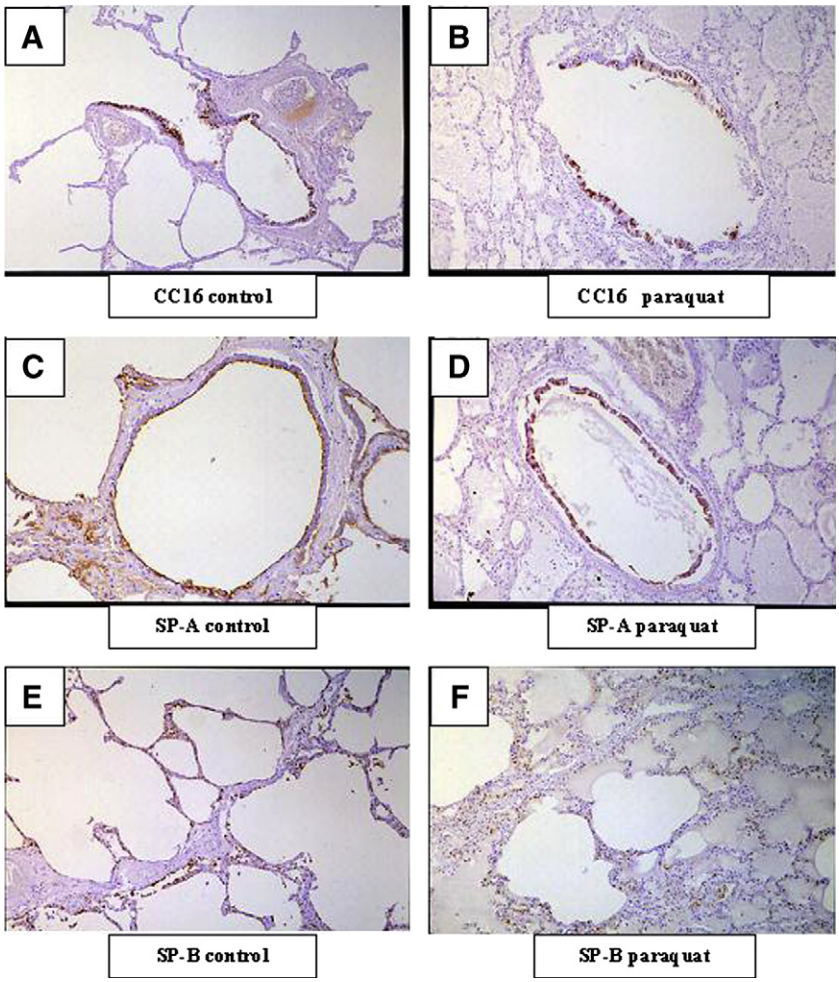


Fig. 3. Immunostained slides of lung tissue from paraquat patient (B, D, F) and control patient (A, C, E) A, B: CC16 immunostaining. No difference in immunostaining was seen between the two specimen. C, D: SP-A immunostaining disappeared in alveolar septa in the paraquat intoxicated lung. E, F: No SP-B was detected in the lung tissue from this patient by opposition to the control.

membranes, typical of diffuse alveolar damage were found. The kidneys showed signs of acute tubular necrosis.

2.1. Determination of biological markers

A broncho-alveolar lavage (BALF) was performed 18 h after admission. The determination of CC16, SP-A and SP-B was made according a previously described technique.[6] The CC16 level in BALF was within normal limits. Serum creatinine began to rise after 30 h together with an increase in serum cystatin-C (Fig. 1). A marked elevation in serum SP-A and SP-B was noticed after 40 h, before any significant change in pulmonary gas exchanges (Fig. 2). The initial serum CC16 concentration was 18 μg/ml and gradually increased until death. No significant correlation was found between serum CC16 and serum creatinine levels.

2.2. Immunostaining examination

Immunostaining with polyclonal antibody CC16 (homemade, 1/20.000 dilution), SPA (Gift from O. Lesur, Canada, 1/10.000 dilution) and SPB (CHEMICON, Temecula, USA, 1/300 dilution) was performed on routinely processed, formalin-fixed, paraffin-embedded sections using standard avidin-biotin peroxidase technique after overnight incubation. These studies were done on postmortem lung tissue specimens and compared to those obtained in a control patient who died from cardiac insufficiency without any lung involvement. CC16 immunostaining was localized in numerous cells of the peripheral bronchi and did not differ between the paraquat patient and the control patient (43% versus 44.5% of positive epithelial cells/total cell count of bronchal epithelium). SP-A immunoreactivity was detected in the peripheral bronchi but not in the alveolar septa. No SP-B immunoreactivity was found following paraquat intoxication (Fig. 3).

3. Discussion

Paraquat is actively concentrated by alveolar and bronchiolar cells (type I and II pneumocytes, Clara cells). This mechanism involves an energy-dependent uptake and is mediated by selective channels for polyamines.[7] Immunostaining analysis in our clinical observation showed that

there was an apparent alteration of cells producing SP-A and SP-B after paraquat exposure (mainly type II pneumocytes). By contrast, Clara cells were less preferentially damaged. This finding is important for the interpretation of the changes of CC16, SP-A and SP-B levels in both BALF and serum because it can be postulated that the cytotoxic effects of paraquat would result in a decreased synthesis and secretion of these proteins in the respiratory tract.

The concentrations of CC16 in BALF of healthy subjects show a great dispersion which suggests inter-individual variation in the synthesis/secretion of CC16 in the respiratory tract.[8–11] CC16 content was significantly decreased in patients with bleomycin-induced lung injury.[12] By contrast, increased CC16 levels have been found in BALF from patients at risk or with full-blown ARDS, the values being on the average higher in survivors than non-survivors.[13] The value we found for CC16 in BALF in the present observation was within normal limits.

Treatment of rats with toxicants that damage Clara cells is usually associated with a decreased level of CC16 in BALF. This has been shown after treatment with ipomeanol (IPO) and methylcyclopentadienyl manganese tricarbonyl (MMT) and to less extent with alpha-naphtylthiourea (ANTU).[14]

In serum, CC16 concentration in humans is much lower than in BALF. Here also, considerable variations have been noted in healthy subjects.[10,11,15] In one study, CC16 was unchanged in the serum of patients with bleomycin-induced lung injury.[11] In the present clinical observation, serum CC16 seemed to markedly increase from 36 to 40 h after exposure, before any detectable changes in pulmonary gas exchanges. The elevation of CC16 in serum was parallel to that of creatinine. Different extrapulmonary factors have been shown to influence the concentration of CC16 in serum. Like other low-molecular-weight proteins, plasma CC16 is rapidly eliminated by glomerular filtration and reabsorbed by the renal tubules. Serum CC16 rises as the glomerular filtration rate declines.[15–17] In the experimental animal model that used IPO, MMT or ANTU to damage Clara cells or endothelial cells, it was shown that the serum CC16 concentration was increased in all treated groups with a pattern of changes quite opposed to that in BALF.[14] As this elevation could not be due to an increase in synthesis or release of the protein in the respiratory tract, it could be explained by the reduction of renal clearance. This hypothesis has to be rejected because none of these toxicants have been reported to be nephrotoxic at the doses used. Another explanation would be an intravascular leakage of CC16 across the broncho-alveolar/blood barrier as assessed by the increase of albumin in BALF in the animals treated by IPO, MMT or ANTU. By contrast to the previous agents, paraquat induces in humans and animals significant nephrotoxicity with reduced glomerular filtration. Therefore, it appears very likely that the changes in serum CC16 were due to the reduction of renal clearance. This is also in accordance with the relative preservation of Clara cells on the immunostained material.

Surfactant-associated proteins have been particularly studied in acute lung injury, a situation known to be associated with alveolar type II cells damage and alterations of the surfactant system. Decreased SP-A levels in BALF and increased SP-A serum levels were observed in patients with ARDS or at risk to develop ARDS.[18–21] Increased SP-A levels in BAL was described in patients with idiopathic pulmonary fibrosis and hypersensitivity pneumonitis.[22] It appeared also that serum SP-B may be increased in patients with ARDS from cardiogenic and non cardiogenic origin.[19,20] The ratio between circulating SP-B and SP-A is approximately 10 as it is also documented by our clinical observation.[23] No specific data are available regarding toxic lung injury. In an animal model, no change in BALF SP-A was noted in rats with bleomycin-induced lung fibrosis.[24] Inconsistent changes have been described with SP-B. Selective downregulation of SP-B was suggested following bleomycin exposure.[25] Surfactant protein D (SP-D) can also be found in alveolar type II cells and bronchiolar cells. The change in serum SP-D was investigated in a rats injured with intraperitoneal paraquat and oxygen.[26] The rats were killed after 1, 2, 3 or 4 week after the first paraquat injection. Serum SP-D levels were increased at each time interval.

In the present observation, the levels of SP-A reported in BALF were in the range of the values observed in patients with newly diagnosed idiopathic pulmonary fibrosis. The increase in BALF SP-B was even more impressive with regard to the values observed in normal volunteers (100 ng/ml).[20]

We hypothesized from the immunostaining studies that the increase of SP-A and SP-B in our paraquat poisoned patient was likely due to a leakage of the pulmonary-vascular barrier. However, it is still necessary to show that renal function had no significant influence on the plasma clearance of SP-A and SP-B as it was suggested by previous publications.[6,27] We showed in a previous paper that, in 54 non-smoking patients with varying degrees of renal dysfunction, serum SP-A and SP-B were not influenced by the impairment of renal function.[28]

There are several limitations to this single observation. The timing for BALF sampling was probably important for the determination of the biological markers. As only one sample could be obtained 19.5 h after exposure, the interpretation of the results should be made with caution. We have also no clear explanation for the transient decrease in the serum SP-A level in the samples obtained, respectively 52 and 58 h after admission. The administration of steroids and cyclophosphamide could have affected the levels of CC16, SP-A and SP-B. The lack of influence of renal failure on SP-A and SP-B excretion still requires further experimental evidence. The immunostaining investigation allows an analysis of qualitative rather than quantitative alterations.

In conclusion, SP-A and SP-B are detectable in serum early after human paraquat exposure. The increase in serum levels may precede the alteration of pulmonary gas

exchanges. The elevation of serum CC16 with paraquat toxicity is probably mainly related to a reduced renal clearance.

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